Published in final edited form as:
Circulation. 2009 August 11; 120(6): 526–532. doi:10.1161/CIRCULATIONAHA.108.841981.

Circulating TGFβ in Marfan's Syndrome

Peter Matt, MD[1,2,3], **Florian Schoenhoff, MD**[1,3], **Jennifer Habashi, MD**[1], **Tammy Holm, MD**[1], **Christel Van Erp, PhD**[1], **David Loch, PhD**[1], **Olga D. Carlson, PhD**[4], **Benjamin F. Griswold, BSc**[4], **Qin Fu, PhD**[3], **Julie De Backer, MD**[5], **Bart Loeys, MD, PhD**[5], **David Huso, PhD**[6], **Nazli B. McDonnell, MD, PhD**[7], **Jennifer E. Van Eyk, PhD**[3], **Harry C. Dietz, MD**[1], and **the GenTAC consortium**

[1]Howard Hughes Medical Institute and Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore [2]Division of Cardiac Surgery, University Hospital, Basel/Berne, Switzerland [3]Johns Hopkins Proteomics Center, National Institute on Aging, Bethesda [4]Laboratory of Clinical Investigation, National Institute on Aging, Bethesda [5]Centre for Medical Genetics, Ghent University, Belgium [6]Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore [7]Clinical Research Branch, National Institute on Aging, Bethesda

Abstract

Background—Marfan syndrome (MFS) is caused by mutations in the fibrillin-1 gene and dysregulation of transforming growth factor β (TGFβ). Recent evidence suggests that losartan, an AT1 blocker that blunts TGFβ activation, may be an effective treatment for MFS. We hypothesized that dysregulation of TGFβ might be mirrored in circulating TGFβ concentrations.

Methods and Results—Serum obtained from MFS mutant mice (*Fbn1*$^{C1039G/+}$) treated with losartan was analyzed for circulating TGFβ1 concentrations, and compared to those from placebo treated and wild-type mice. Aortic root size was measured by echocardiography. Data was validated in patients with MFS and healthy individuals. In mice, circulating total TGFβ1 concentrations increased with age and were elevated in older untreated *Fbn1*$^{C1039G/+}$ mice compared to wild-type mice (P=0.01; n=16, mean±SEM 115±8 ng/ml vs. n=17, 92±4 ng/ml). Losartan-treated *Fbn1*$^{C1039G/+}$ mice had lower total TGFβ1 concentrations compared to age-matched *Fbn1*$^{C1039G/+}$ mice treated with placebo (P=0.01; n=18, 90±5 ng/ml), and circulating total TGFβ1 levels were indistinguishable from those of age-matched wild-type mice (P=0.8). Correlation was observed between circulating TGFβ1 levels and aortic root diameters in *Fbn1*$^{C1039G/+}$ and wild-type

Corresponding authors: Jennifer Van Eyk, PhD 602 Mason F. Lord Bldg, Center tower Johns Hopkins University Baltimore, MD 21239 Tel. 410-550-8510 Fax.410-550-8512 jvaneyk1@jhmi.edu. Peter Matt, MD Division of Cardiac Surgery University Hospital CH-4031 Basel Tel. 0041-61-265-2525 Fax. 0041-61-265-8854 pmatt@uhbs.ch.

GenTAC (National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions) participating centers:
Johns Hopkins University Kathryn W. Holmes, MD Harry C. Dietz, MD Williams Ravekes, MD Kira Lurman, RN
University of Texas – Houston Dianna M. Milewicz, MD, PhD Claire Noll, MS, CGC
Baylor College of Medicine Scott A. LeMaire, MD Irina Volguina, PhD
Oregon Health & Science University Cheryl L Maslen, PhD Howard K. Song, MD, PhD Victor Menashe, MD Jessica D. Kushner, MS, CGC
University of Pennsylvania Reed E. Pyeritz, MD, PhD Joseph E. Bavaria, MD Megan Morales
Weill Medical College of Cornell University Craig T. Basson, MD, PhD Richard Devereux, MD Jonathan W. Weinsaft, MD Deborah McDermott, MS, CGC
University of Michigan Kim Eagle, MD
National Heart, Lung, and Blood Institute H. Eser Tolunay, PhD Patrice Desvigne-Nickens, MD Mario P. Stylianou, PhD Megan Mitchell, MPH
RTI International Barbara L. Kroner, PhD Donald Brambilla, PhD Tabitha Hendershot Danny Ringer Meg Cunningham Mark Kindem

Disclosures See funding sources, no others.

mice (P=0.002). In humans, circulating total TGFβ1 concentrations were elevated in patients with MFS compared to control individuals ($P<0.0001$; n=53, 15±1.7 ng/ml vs. n=74, 2.5±0.4 ng/ml). MFS patients treated with losartan (n=55) or β-blocker (n=80) showed significantly lower total TGFβ1 concentrations compared to untreated Marfans ($P\leq0.05$).

Conclusions—Circulating TGFβ1 concentrations are elevated in MFS, decrease after administration of losartan and/or β-blocker therapy, and therefore might serve as prognostic and therapeutic marker in MFS.

Keywords

Marfan; TGFβ; Aneurysm; Losartan; Biomarker

Marfan syndrome (MFS) is a systemic connective tissue disorder that affects approximately 1 in 5,000 individuals[1,2]. It is inherited as an autosomal dominant trait and is caused by mutations in the gene encoding the extracelluar matrix protein fibrillin-1 (*FBN1*)[3]. *FBN1* mutations lead to defects in multiple organ systems. Aortic root dilatation is the leading cause of morbidity and mortality in MFS[1,4]. Several studies, mainly based on the analysis and creation of genetically engineered mouse models, have challenged the definition of MFS as a simple structural disorder of the connective tissue[5–8]. It has been shown that many clinical manifestations associated with MFS including aortic root dilatation, pulmonary emphysema, atrioventricular valve changes and skeletal muscle myopathy are induced by altered TGFβ activation and signaling[5–7,9]. A mouse model heterozygous for a mutant *Fbn1* allele encoding a cysteine substitution in an epidermal growth factor-like domain of fibrillin-1, $Fbn1^{C1039G/+}$, was shown to develop pathologic changes in the aorta and aortic root enlargement that closely mimic those seen in humans with MFS accompanied by excessive TGFβ signaling in the aortic root wall[7]. Most importantly, TGFβ antagonism through systemic administration of TGFβ neutralizing antibody (NAb) prevented the development of pathologic changes in the aortic wall and progressive aortic dilatation[7]. TGFβ antagonism also rescued other manifestations of MFS, including muscle regeneration, architecture and strength, pulmonary alveolar septation, and mitral valve morphology in the mouse model[5,8]. Administration of losartan, an angiotensin II type 1 (AT1) receptor blocker (ARB) known to antagonize TGFβ signaling through inhibition of TGFβ expression and activation[10], normalized aortic root growth and dimensions in the $Fbn1^{C1039G/+}$ mice, and resulted in an aortic wall architecture that was indistinguishable from wild-type mice[7]. The therapeutic benefit of losartan was recently replicated in a small pediatric cohort with a severe form of MFS[11]. Losartan, either alone or in addition to β-blocker therapy led to a reduction in the rate of change in the aortic root diameter as compared with beta-blocker therapy alone[11]. Taken together, these data suggest that dysregulation of TGFβ, which is an autocrine and paracrine growth factor with involvement in a wide range of biological processes, contributes to the multisystem pathogenesis of MFS[4,7,11].

TGFβ is secreted from the cell in the context of a large latent complex (LLC) that includes the active cytokine, its processed N-terminal propetide (called latency associated peptide or LAP) and one of three latent TGFβ binding proteins (LTBP1, 3 or 4). The LLC is targeted to the matrix by virtue of interactions between LTBPs and both fibronectin and fibrillins. Current models suggest that fibrillin-1 deficiency leads to failed matrix sequestration of the large latent complex of TGFβ, with consequent excessive TGFβ activation and signaling. In this light, pathogenic events might correlate with circulating TGFβ concentrations. If so, then systemic administration of losartan in the mouse models of MFS should decrease circulating TGFβ concentrations. If transferable to humans, circulating TGFβ might serve as a promising marker for prognostication and individualizing therapeutic regimens in MFS.

Methods

All mouse and human protocols were approved by the institutional review board of Johns Hopkins University School of Medicine, and MedStar Research Institute, Baltimore, Maryland, USA.

Mice

The mouse line heterozygous for *Fbn1* mutation C1039G (*Fbn1*$^{C1039G/+}$) has been previously described[5,6]. All analyses were performed after back-crossing this mutation into the C57BL/6J background (>9 generations), allowing valid comparisons between litters. *Fbn1*$^{C1039G/+}$ mice were treated with oral losartan (0.6 g/l in drinking water; n=18) or placebo (n=37), beginning at 7 weeks of age and continued until they were sacrificed; wild-type littermates received only placebo (n=41). A small subgroup of *Fbn1*$^{C1039G/+}$ mice was treated with a higher dose of oral losartan (1.2 g/l in drinking water; n=3). All mice were sacrificed with an inhalation overdose of halothane (Sigma-Aldrich, St. Louis). Blood samples from mice were collected from the right ventricle immediately after they were sacrificed, and were allowed to clot for 2 hours at room temperature before being centrifuged for 20 min at 2000 *g*. Serum was removed, aliquotted and immediately stored at −80°C until further analysis.

Human Subjects

Plasma samples and clinical data from 207 patients diagnosed with Marfan Syndrome were collected through the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC). Plasma samples from 74 healthy individuals obtained at the National Institute on Aging (NIA) served as controls. Selection criteria for control patients were: no cardiovascular disease or drugs, no cancer, no inflammatory of autoimmune disorders, no diabetes and no drugs that could affect the extracellular matrix. Blood samples in MFS and control patients were collected on ice using EDTA as an anticoagulant. Samples were centrifuged at 3000 *g* for 15 min at 4°C, and plasma samples immediately stored at −80°C until further analysis.

ELISA for TGFβ

TGFβ1 concentrations in mice samples were measured by ELISA using the Quantikine TGFβ1 immunoassay (from R&D Systems, Minneapolis, MN, catalog no. MB100M). The minimum detectable concentration of TGFβ1 in this assay ranges from 1.7 to 15.4 pg/ml and there is less than 1% cross-reactivity with the latent TGFβ1 complex. Total TGFβ1 concentrations were measured by first acid activating (with 1N HCl) latent TGFβ1 to immunoreactive TGFβ1, free (active) TGFβ1 levels were measured without first acid activating the sample. The ELISA immunoassay was performed according to the manufacturer`s protocol. Briefly, the wells are pre-coated with monoclonal antibody specific for TGFβ1. Standards, controls and samples were added and incubated for 2 h. After washing an enzyme-linked polyclonal antibody specific for TGFβ1 was added. After another washing, samples were incubated with a substrate solution for 30 min and the color reaction stopped by addition of a diluted HCl solution. The optical density of each well was measured immediately with a microplate reader at 450 nm and the wavelength correction was set to 570 nm. All samples were run in duplicate.

Human EDTA plasma samples were assayed for levels of total TGFβ1 using a commercially available, Ruthenium based electrochemoluminescence platform (Meso Scale Discovery, Gaithersburg, MD) following the manufacturer`s recommendations. In order to be able to measure total TGFβ1, samples were first acid-activated (with 1N HCl) before assaying. All samples were run in duplicate. The lowest level of quantification for total TGFβ1 was 4±2.6 pg/ml.

We considered TGFβ1 results (in mice and in human samples) valid when recovery (expected concentration divided by calculated concentration multiplied by 100) of the standards/ calibrators was 100±20%, the coefficient of variation was <20%, intra-assay CV was <10% and the interassay CV was <20%. A run was considered valid when >85% of the samples were within these specifications.

Aortic root diameters: Echocardiography

Transthoracic echocardiograms (TTE) (n=39) were performed on awake, unsedated mice using the VisualSonics Vevo 660 V1.3.6 imaging system and a 40 or 60-MHz transducer (model RMV603, VisualSonics, Inc., Toronto). The aorta was imaged in the parasternal long axis view, and 3 measurements were obtained at the level of the sinuses of Valsalva (SOV) by an observer blinded to genotype and treatment arm. All echocardiographic studies were performed by 2 individuals with extensive experience with mouse echocardiography.

In patients with MFS, aortic root dimensions (SOV) were obtained through TTE by multiple echocardiographers. All echocardiographic data were collected in the GenTAC registry before measurements of circulating TGFβ1 concentrations were performed. Measurements of the maximal aortic root diameter were taken by the leading edge technique, consistent with the current American Society of Echocardiography guidelines[12]. A z-score, which represents the standard deviation from the mean aortic diameter normalized for the patient`s body-surface area and age, was calculated from each echocardiographic measurement using standard algorithms.

Statistical analysis

Continuous variables were presented as the mean±SEM. Comparisons of circulating TGFβ1 concentrations among the groups were conducted by one-way ANOVA. If significance was found for group effect, pairwise comparisons between the groups were made using the unpaired *t* test. Associations between circulating TGFβ1 concentrations and SOV diameters were studied using the Pearson correlation and linear regression analysis, and illustrated in scatterplots with confidence bands on a regression line. For comparisons of nonparametric data the Chi-squared test was used. Two-sided *P* values of less than 0.05 were considered to indicate statistical significance for all statistical tests and models. SPSS statistical software 9.0 was used for all analyses.

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Mice

One-way ANOVA showed high group effects for circulating total and free TGFβ1 concentrations (P=0.002, P<0.0001). Circulating total TGFβ1 concentrations increased with age in $Fbn1^{C1039G/+}$ and wild-type mice (Figure 1A, 1B). Total TGFβ1 was higher in samples obtained from 6 to 10 month old $Fbn1^{C1039G/+}$ mice compared to age-matched wild-type mice (P=0.01; n=16, mean±SEM 115±8 ng/ml vs. n=17, 92±4 ng/ml; Figure 1A). Losartan-treated $Fbn1^{C1039G/+}$ mice had lower total TGFβ1 concentrations compared to age-matched $Fbn1^{C1039G/+}$ mice treated with placebo (P=0.01; n=18, 90±5 ng/ml vs. n=16, 115±8 ng/ml; Figure 1A). Circulating total TGFβ1 in losartan-treated $Fbn1^{C1039G/+}$ mice and age-matched wild-type mice were indistinguishable (P=0.8; Figure 1A). A subgroup of $Fbn1^{C1039G/+}$ mice (n=3) treated with a higher dose of losartan (1.2 g/l in drinking water) showed a further

reduction of circulating TGFβ1 concentrations (Figure 1A). Changes in circulating free TGFβ1 levels mirrored the changes seen in total TGFβ1 (Figure 1B). Correlation was observed between circulating total TGFβ1 and free TGFβ1 concentrations in matched samples (n=43; r=0.6, P<0.0001).

Aortic root diameters (SOV) were smaller in 6 to 10 months old wild-type mice compared to age-matched untreated *Fbn1*$^{C1039G/+}$mice (P<0.0001; mean±SEM n=15, 1.69±0.02 mm vs. n=10, 2.23±0.11 mm). Losartan treatment in *Fbn1*$^{C1039G/+}$ mice led to smaller SOV dimensions compared to age-matched untreated *Fbn1*$^{C1039G/+}$ mice (P<0.0001), and losartan-treated *Fbn1*$^{C1039G/+}$ mice and age-matched wild-type mice showed indistinguishable SOV dimensions, as shown previously[7]. SOV diameters in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice correlated with circulating total TGFβ1 (r=0.6, P=0.002) and circulating free TGFβ1 concentrations (r=0.6, P<0.001; Figure 1C, 1D).

Human Subjects

Baseline demographics for patients diagnosed with MFS (n=207) and control individuals (n=74) are provided in Table 1. Marfan patients were younger and there were more males within the MFS group compared to the controls. Previous aortic root surgery and cardiovascular drug therapy including losartan, other ARBs, β-blocker and angiotensin converting enzyme (ACEi) inhibitors occurred only in people with MFS.

One-way ANOVA showed high group effects for circulating total TGFβ1 concentrations (P<0.0001). Circulating total TGFβ1 concentrations were elevated in MFS patients without cardiovascular drug therapy compared to the control individuals (P<0.0001; n=53, mean±SEM 15±1.7 ng/ml vs. n=74, 2.5±0.4 ng/ml; Figure 2A). Marfan patients treated with losartan (n=55, of those 45 patients received a combination of losartan and β-blocker therapy) showed significantly lower total TGFβ1 concentrations compared to non-treated Marfan patients (P=0.05; 11±1.4 ng/ml vs. 15±1.7 ng/ml; Figure 2A). Similarly, total TGFβ1 concentrations were lower in MFS patients treated only with β-blockers (P=0.03; n=80, 11±1.2 ng/ml vs. 15 ±1.7 ng/ml; Figure 2A). A subgroup of MFS patients treated with ACE inhibitors (n=12, of those 10 patients received a combination of ACEi and β-blocker therapy) showed a tendency towards lower total TGFβ1 concentrations (P=0.1; 12.7±2.8 ng/ml vs. 15±1.7 ng/ml; Figure 2A). Marfan patients with either losartan, β-blocker or ACE inhibitor therapy showed still significantly higher circulating TGFβ1 levels compared to the control individuals (P<0.0001). Correlations between circulating total TGFβ1 concentrations and SOV diameters or z-scores, respectively, in Marfan patients were not significant (Figure 2B). Circulating total TGFβ1 concentrations were neither gender nor age dependent in people with MFS and healthy control individuals (Figure 2C, 2D).

Discussion

Recent studies have established the critical contribution of dysregulated TGFβ signaling to the progression of disease in MFS[5–8]. Mouse models have validated TGFβ antagonism as a productive treatment strategy for both the cardiovascular and systemic manifestations of MFS, including the use of the losartan, and early experience suggests that this protection may extend to people with MFS[7,11]. Despite significant progress regarding the pathogenesis and treatment of MFS, many obstacles remain. In a small observational study of children with severe MFS treated with losartan, all showed at least some reduction in aortic root growth when compared to their progression on prior medical therapy, but the degree of response was variable[11]. While widespread adoption of the practice of performing aortic root surgery once the dimension exceeds 5.0 cm in adults has greatly reduced mortality due to aortic dissection, meaningful guidelines for children are lacking and death due to root dissection continues to be observed in both age groups. Even among patients with successful prophylactic aortic root replacement,

there is risk of dissection of other aortic segments, prominently of the proximal descending thoracic aorta, and this occurs in many patients without prior dilatation.

In the absence of empiric data, the dose of losartan or other medications currently used in patients with MFS is largely based on dosing regimens for hypertension. While the weight-based dosing for losartan used in mice was considerably higher than that for people, metabolic differences between species preclude extrapolation[7]. Consideration of dosing may be particularly important, since ultrahigh doses of ARBs and other medications have been suggested to have enhanced efficacy in treating other TGFβ-related diseases in both mice and people[7,8,11]. Furthermore, natural genetic variation in people, including genes encoding regulators of drug metabolism or the renin-angiotensin system (RAS), can influence both the effective level of and intrinsic response to ARBs, ACEi and β-blockers. A similar “one size fits all” philosophy for surgical management is intuitively limited. Taken together, these observations highlight the need for an informative prognostic and/or therapeutic marker in MFS. The lack of robust phenotype-genotype correlations in MFS suggests that *FBN1* genotype fails to accommodate this need.

Despite gaps in our understanding of the precise mechanism by which fibrillin-1 deficiency correlates with increased TGFβ signaling, current data are consistent with a model in which LLC that fails to be sequestered by the extracellular matrix is more bioavailable for or prone to activation in a protease-dependent (e.g. matrix metalloproteinases (MMPs) or plasmin) or independent (e.g. through the action of thrombospondin-1 or selected integrins) manner[13–16]. Curiously, the levels of selected TGFβ activators (including MMP2, MMP9 and thrombospondin-1) and ligands have been shown to be elevated in the tissues of patients with MFS, including the aortic wall[17]. It remains to be determined whether this is the result of positive autoregulation by TGFβ or either proximal or parallel events. Nevertheless, blockade of the angiotensin II type 1 receptor with ARBs has been shown to diminish the expression of TGFβ, its receptor and potential activators including thrombospondin-1 and MMPs, providing multiple potential mechanisms of protection from TGFβ-induced pathogenic events[7]. In keeping with this paradigm, we now report that a genetically-defined mouse model of MFS that faithfully recapitulates most aspects of the disease including progressive aortic root dilatation shows elevated circulating levels of both latent and active TGFβ. Furthermore, circulating TGFβ levels show close correlation with aortic root dimension in mice that have or have not been treated with losartan, diminish upon treatment in a dose-dependent manner, and are fully normalized in mice that show a robust therapeutic response.

While the use of circulating TGFβ levels to individualize patient counseling and management is appealing since the assay is monitoring a central and direct effector of disease progression, it is essential to gain a better understanding of how and why events in tissues and markers in the circulation correlate. The simplest hypothesis posits that complexed and free TGFβ simply leach into the circulation due to failed matrix sequestration and increased activation of the LLC, respectively. It is also formally possible that increased circulating active TGFβ contributes to altered cellular and tissue performance. This seems unlikely, however, given the relatively high pericellular and matrix concentrations of TGFβ that result from direct secretion, when compared to that in the circulation. The description of a patient with hemi-MFS, as a result of discrete lateral somatic mosaicism, also argues against a major contribution of circulating TGFβ to the disease phenotype[18].

In keeping with our results, the ability of ARBs to lower circulating TGFβ levels has been observed in other disease processes, and perinopril was shown to lower elevated circulating TGFβ levels in a small group of patients with MFS[19]. While we did observe a significant increase in the amount of total TGFβ in MFS patients versus controls, the levels of free TGFβ were extremely low in both groups, precluding a meaningful comparison. This is not

surprising since human latent TGFβ1 has a half-life of approximately 90 minutes, whereas free TGFβ1 has a half-life of only 2 minutes[14,15]. It is possible that modified handling or processing of human samples would allow for valid measurements.

It is intriguing that Marfan patients treated with β-adrenergic receptor blocking agents (β-blockers) showed a significant decrease in circulating levels of TGFβ. While prior studies have linked β-adrenergic and TGFβ signaling, the mechanistic details are not well established. Patients with dilated cardiomyopathy-associated fibrosis showed decreased expression of TGFβ1 and its target genes encoding types I and III collagens upon treatment with β-blockers[20]. Treatment of Marfan mouse models with atenolol reduced TGFβ expression and signaling, although this effect appeared restricted to early age groups[21]. Inkeeping with this finding, Marfan mice treated with propranolol showed a significant reduction in aortic root size compared to placebo-treated animals. However, there was no apparent effect on TGFβ signaling in the aortic wall in older animals, and the extent of chronic protection was far less than that achieved with losartan[7]. The finding that TGFβ can positively stimulate its own expression and activation and can increase beta-adrenergic receptor density and signaling in the cardiovascular system[22] highlights the potential for both auto-and cross-induction of these signaling cascades. The extent to which circulating TGFβ levels serve as a valid surrogate for critical pathogenic events in the tissues of Marfan patients may prove both dynamic and context-dependent. The emerging view is that β-blockers can reduce TGFβ expression while ARBs reduce both expression and activation. In this light, the reduction in circulating TGFβ seen while on β-blockers or ARBs may herald protection in tissues or stages in disease progression where TGFβ signaling is limited by TGFβ expression levels. In other contexts, where excessive TGFβ activation is sufficient to achieve pathogenic signaling thresholds, the prognostic value of a drop in circulating TGFβ levels may be unique to patients receiving ARBs. This important issue clearly warrants further study. We did not observe close correlation between circulating TGFβ and aortic root size or z-score in people with MFS. There are many possible explanations. First, surrogate markers in human samples often show a much higher variability than seen in mice with the same genetic background and standardized environment. Second, our study mainly focused on adults, and was enriched for people with milder disease and who were receiving medical therapy that could modify pathogenic events including TGFβ levels. Finally, the aortic root is likely a minor contributor to circulating TGFβ levels, when compared to other tissues that are affected in MFS including skeletal muscle, skin and lung. While there is high concordance between the severity of aortic and systemic disease in the various inbred mouse strains with *Fbn1* mutations and in people at the extremes of disease severity, this is not the case in people with more moderate presentations of MFS. In this light, the high correlation between circulating TGFβ levels and aortic performance in mice might be recapitulated in defined subsets of patients. Even if there is little intrinsic prognostic value to a snapshot measurement of circulating TGFβ in people in MFS, there remains high probability of informativeness for individual trends observed during the progression of disease and/or in response to therapy.

Acknowledgments

We thank the GenTAC investigators, collaborators and coordinators. Peter Matt thanks the Swiss National Foundation, the Novartis Foundation and the Hippocrate Foundation Basel for financial support. Florian Schoenhoff thanks the Swiss National Foundation and the Novartis Foundation for financial support. Bart Loeys and Julie De Backer are Senior Clinical Investigators of the Fund for Scientific Research Flanders. Nazli McDonnell thanks Leslie Sloper, NIA-ASTRA unit staff and NIA core lab for facilitation of sample collection and processing. We thank Hendrik Tevaearai, MD, and Thierry Carrel, MD, Inselspital Berne, Switzerland, for critical reading and editing of the manuscript. We thank Brigitta Gahl, Inselspital Berne, for support in statistical analyses.

Funding Sources Peter Matt is supported by the Swiss National Foundation, the Novartis Foundation and the Hippocrate Foundation Basel. Jennifer E. Van Eyk is supported by grants from the National Heart, Lung, and Blood Institute Proteomic Initiative (contract NO-HV-28120), the Daniel P. Amos Family Foundation and the Institute for

Clinical and Translational Science Award (Grant NO 1U54RR023561-01A1). Harry C. Dietz is supported by the NIH, the Howard Hughes Medical Institute, the William S. Smilow Center for Marfan Syndrome Research, and the National Marfan Foundation. Nazli McDonnell, Ben Griswold and Olga Carlson are supported by intramural funds originating at the NIA/NIH. Florian Schoenhoff is supported by the Swiss National Foundation and the Novartis Foundation.

References

1. Judge DP, Dietz HC. Marfan's syndrome. Lancet 2005;366:1965–76. [PubMed: 16325700]
2. Pearson GD, Devereux R, Loeys B, Maslen C, Milewicz D, Pyeritz R, Ramirez F, Rifkin D, Sakai L, Svensson L, Wessels A, Van Eyk J, Dietz HC. Report of the National Heart, Lung, and Blood Institute and National Marfan Foundation Working Group on research in Marfan syndrome and related disorders. Circulation 2008;118:785–91. [PubMed: 18695204]
3. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Francomano CA. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991;352:337–9. [PubMed: 1852208]
4. Matt P, Habashi J, Carrel T, Cameron DE, Van Eyk JE, Dietz HC. Recent advances in understanding Marfan syndrome: should we now treat surgical patients with losartan? J Thorac Cardiovasc Surg 2008;135:389–94. [PubMed: 18242274]
5. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 2003;33:407–11. [PubMed: 12598898]
6. Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, Gabrielson KL, Hausladen JM, Mecham RP, Judge DP, Dietz HC. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J Clin Invest 2004;114:1586–92. [PubMed: 15546004]
7. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, Dietz HC. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006;312:117–21. [PubMed: 16601194]
8. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007;13:204–10. [PubMed: 17237794]
9. Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, Sakai LY, Dietz HC. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. J Clin Invest 2004;114:172–81. [PubMed: 15254584]
10. Lavoie P, Robitaille G, Agharazii M, Ledbetter S, Lebel M, Lariviere R. Neutralization of transforming growth factor-beta attenuates hypertension and prevents renal injury in uremic rats. J Hypertens 2005;23:1895–903. [PubMed: 16148614]
11. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC 3rd. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med 2008;358:2787–95. [PubMed: 18579813]
12. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440–63. [PubMed: 16376782]
13. Cox DA, Maurer T. Transforming growth factor-beta. Clin Immunol Immunopathol 1997;83:25–30. [PubMed: 9073531]
14. Gleizes PE, Munger JS, Nunes I, Harpel JG, Mazzieri R, Noguera I, Rifkin DB. TGF-beta latency: biological significance and mechanisms of activation. Stem Cells 1997;15:190–7. [PubMed: 9170210]
15. Mangasser-Stephan K, Gressner AM. Molecular and functional aspects of latent transforming growth factor-beta binding protein: just a masking protein? Cell Tissue Res 1999;297:363–70. [PubMed: 10460484]

16. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 1999;96:319–28. [PubMed: 10025398]

17. Segura AM, Luna RE, Horiba K, Stetler-Stevenson WG, McAllister HA Jr. Willerson JT, Ferrans VJ. Immunohistochemistry of matrix metalloproteinases and their inhibitors in thoracic aortic aneurysms and aortic valves of patients with Marfan's syndrome. Circulation 1998;98:II331–7. [PubMed: 9852923]

18. Burgio RG, Martini A, Cetta G, Zanaboni G, Vitellaro L, Danesino C. Asymmetric Marfan syndrome. Am J Med Genet 1988;30:905–9. [PubMed: 3189412]

19. Ahimastos AA, Aggarwal A, D'Orsa KM, Formosa MF, White AJ, Savarirayan R, Dart AM, Kingwell BA. Effect of perindopril on large artery stiffness and aortic root diameter in patients with Marfan syndrome: a randomized controlled trial. Jama 2007;298:1539–47. [PubMed: 17911499]

20. Shigeyama J, Yasumura Y, Sakamoto A, Ishida Y, Fukutomi T, Itoh M, Miyatake K, Kitakaze M. Increased gene expression of collagen Types I and III is inhibited by beta-receptor blockade in patients with dilated cardiomyopathy. Eur Heart J 2005;26:2698–705. [PubMed: 16204268]

21. Chung AW, Yang HH, Radomski MW, van Breemen C. Long-term doxycycline is more effective than atenolol to prevent thoracic aortic aneurysm in marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9. Circ Res 2008;102:e73–85. [PubMed: 18388324]

22. Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U, Schluter KD, Bohm M. Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1). Am J Physiol Heart Circ Physiol 2002;283:H1253–62. [PubMed: 12181157]

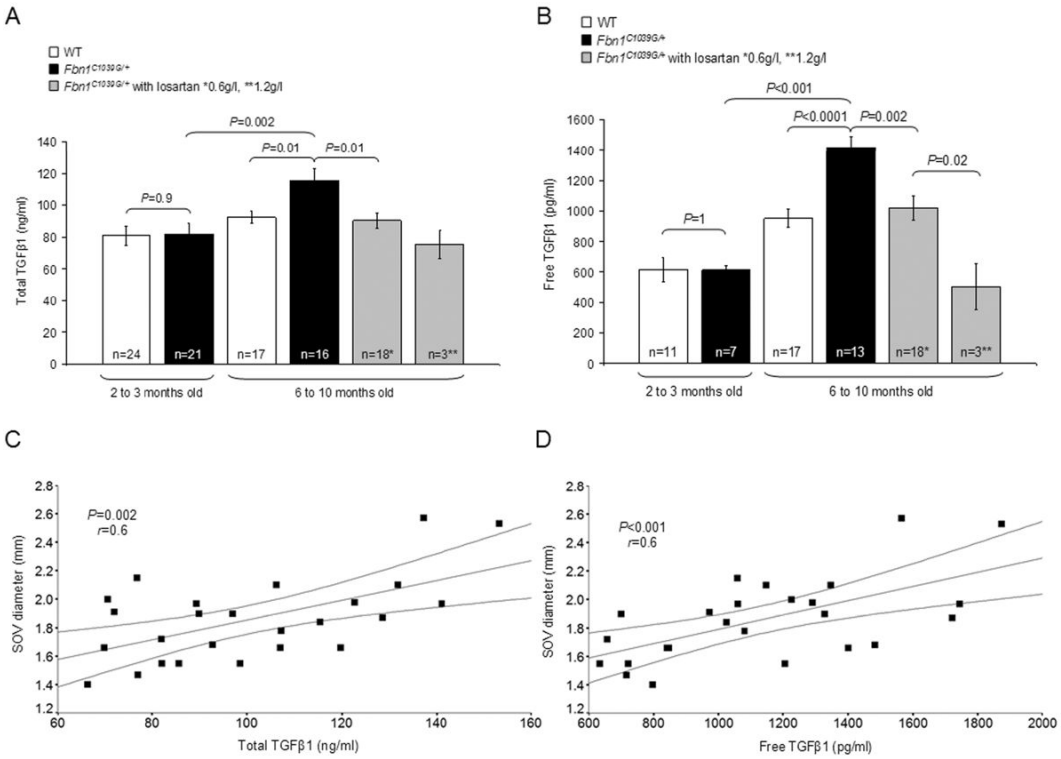

Figure 1.

Circulating TGFβ1 concentrations in *Fbn1*$^{C1039G/+}$ and wild-type mice and their correlation with the aortic root size. **(A)** Mean total TGFβ1 serum concentrations showing increased concentrations with age, and significantly higher circulating total TGFβ1 levels in 6 to 10 month old *Fbn1*$^{C1039G/+}$ mice compared to age-matched wild-types and losartan-treated *Fbn1*$^{C1039G/+}$ mice. **(B)** Mean free TGFβ1 serum concentrations changed in the same manner as seen with total TGFβ1. A subgroup of *Fbn1*$^{C1039G/+}$ mice was treated with a higher dose of losartan (1.2 g/l in drinking water instead of the 0.6 g/l standard dose) which resulted in a further reduction in circulating total and free TGFβ1 concentrations. **(C)** Correlation was observed between circulating total TGFβ1 concentrations and the sinus of Valsalva (SOV) diameters in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice (regression prediction lines with mean value and CI 95%). **(D)** Correlation was observed between free TGFβ1 concentrations and SOV size in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice (regression prediction lines with mean value and CI 95%).

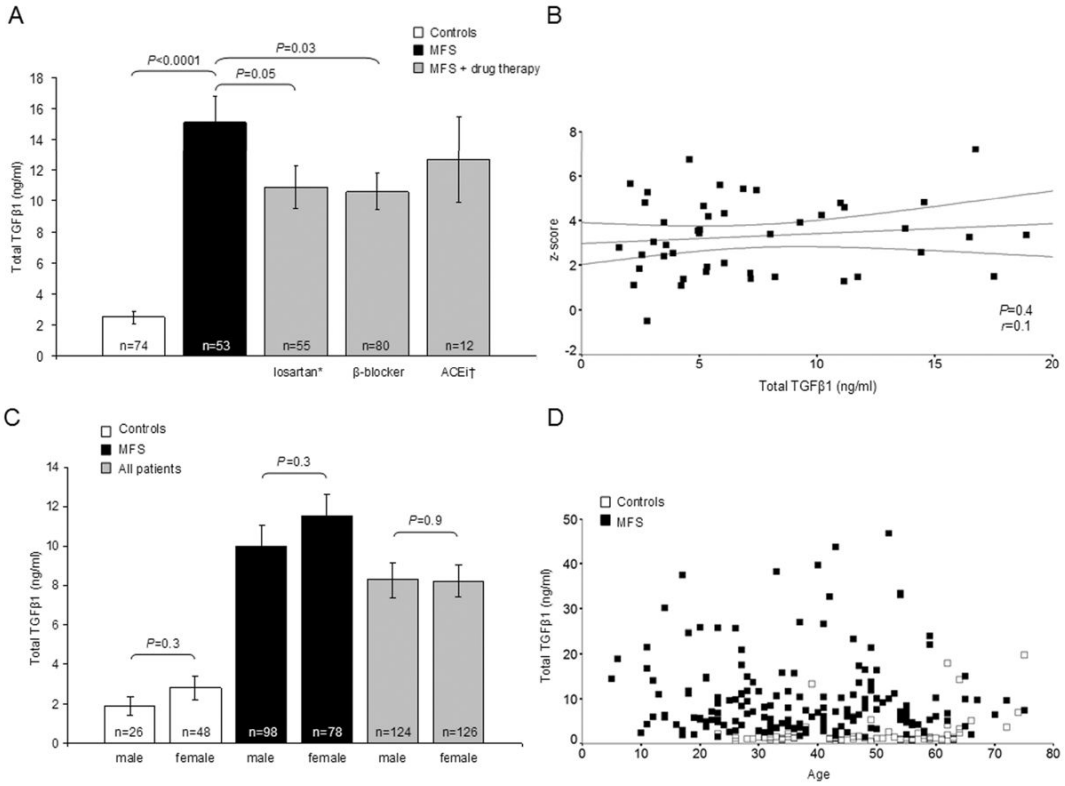

Figure 2.

Circulating total TGFβ1 concentrations in human samples, their correlation with z-scores in MFS patients, and the association with gender and age, respectively. **(A)** Mean total TGFβ1 plasma concentrations were elevated in MFS patients without cardiovascular drug therapy compared to healthy control individuals. MFS patients with losartan and/or β-blocker treatment showed a significant decrease in circulating total TGFβ1 levels compared to non-treated Marfans, and those with an ACEi therapy showed a tendency towards lower total TGFβ1 concentrations. *45 patients with combined losartan and β-blocker therapy; †10 patients with combined ACEi and β-blocker therapy **(B)** Correlation between circulating total TGFβ1 concentrations and z-scores in MFS patients without previous aortic root surgery was not significant (regression prediction lines with mean value and CI 95%). In addition, no significant correlation was observed in MFS patients without previous aortic surgery and no cardiovascular drug therapy (n=12, r=0.1, P=0.7). **(C)** Mean total TGFβ1 concentrations showed no significant difference between male and female in healthy control individuals and people with MFS. **(D)** No correlation was observed between total TGFβ1 concentrations and age in controls and those with MFS (r=0.3; r=0.07).

Table 1

Patients baseline demographics

Variables	Marfans *n=207*	Controls *n=74*	
Age (years)*	37.5 (±1.2)	48.7 (±1.5)	*P*<0.0001
Gender (male:female)	98:78	26:48	*P*=0.003
BMI*	24.6 (±0.5)	25 (±0.4)	*P*=0.6
Aortic root dimension*†			
SOV (cm)	3.8 (±0.1)	-	
z-score‡	3.2 (±0.2)	-	
Previous aortic root surgery	77 (37%)	0	
No cardiovascular drug therapy	53 (25%)	74 (100%)	
Medication			
Losartan§	55 (26%)	0	
β-blockers	80 (39%)	0	
ACE inhibitors//	12 (7%)	0	
Others#	7 (3%)	0	

Abbreviations: BMI=body mass index; SOV=sinus of Valsalva; ACE=angiotensin converting enzyme;

*data are presented as mean±SEM;

†data from patients without previous aortic root surgery;

‡represents the standard deviation from the mean aortic diameter normalized for the patient's body-surface area and age;

§45 patients with combined losartan and β-blocker therapy;

//10 patients with combined ACEi and β-blocker therapy;

#other ARB therapy than losartan, calcium channel blocker or combination of losartan, ACEi and β-blocker.